

CLAIMS

I claim:

1. A method for producing a nucleic acid molecule that comprises a continuous nucleotide sequence of interest derived from noncontiguous nucleotide sequences, comprising:

(a) amplifying at least two nucleotide sequences from a nucleic acid molecule template using primer pairs to produce double-stranded amplified products, wherein the amplified nucleotide sequences reside noncontiguously in the nucleic acid molecule template, wherein each primer of a primer pair comprises a continuous recognition sequence for a class IIS restriction endonuclease which is located near the 5'-end of the primer, such that cleavage of the amplified products with the class IIS restriction endonuclease yields at least two nucleic acid molecule fragments with cohesive ends that, when ligated to each other, produce a continuous nucleotide sequence of interest,

(b) cleaving amplified products with the class IIS restriction endonuclease to produce nucleic acid molecule fragments, and

(c) ligating cleaved nucleic acid molecule fragments to produce a nucleic acid molecule comprising the continuous nucleotide sequence of interest.

2. A method for producing a nucleic acid molecule that comprises a continuous nucleotide sequence of interest derived from noncontiguous nucleotide sequences, comprising:

(a) amplifying at least two nucleotide sequences from at least two nucleic acid molecule templates using primer pairs to produce double-stranded amplified products, wherein each primer of a primer pair comprises a continuous recognition sequence for a class IIS restriction endonuclease which is located near the 5'-end of the primer, such that cleavage of the amplified products with the class IIS restriction endonuclease yields at least two nucleic acid molecule fragments with cohesive ends that, when ligated to each other, produce a continuous nucleotide sequence of interest,

(b) cleaving amplified products with the class IIS restriction endonuclease to produce nucleic acid molecule fragments, and

(c) ligating cleaved nucleic acid molecule fragments to produce a nucleic acid molecule comprising the continuous nucleotide sequence of interest.

3. The method of claim 1, wherein the class IIS restriction endonuclease recognizes a five-base continuous recognition sequence.

4. The method of claim 3, wherein the class IIS restriction endonuclease is selected from the group consisting of *AcIWI*, *Alw26I*, *AlwI*, *AsuHPI*, *BbvI*, *BceII*, *BinI*, *BseGI*, *BseMII*, *BseXI*, *BspPI*, *BsmAI*, *Bst71I*, *BstF5I*, *FauI*, *FokI*, *HgaI*, *HphI*, *MboII*, *PleI*, *SfaNI*, and *TspRI*.

5. The method of claim 1, wherein the class IIS restriction endonuclease recognizes a six-base continuous recognition sequence.

6. The method of claim 5, wherein the class IIS restriction endonuclease is selected from the group consisting of *AceIII*, *BbsI*, *BbvII*, *Bce83I*, *BciVI*, *BfiI*, *BfuI*, *BmrI*, *BpiI*, *BpmI*, *BpuAI*, *BsaI*, *Bse3DI*, *BseRI*, *BsgI*, *BsmBI*, *BsmFI*, *BspMI*, *BsrDI*, *Bsu6I*, *Eam1104I*, *EarI*, *Eco31I*, *Eco57I*, *Esp3I*, *FauI*, *GsuI*, *Ksp632I*, *MmeI*, *RleAI*, *TaqII*, and *Tth111II*.

7. The method of claim 1, wherein the class IIS restriction endonuclease recognizes a seven-base continuous recognition sequence.

8. The method of claim 7, wherein the class IIS restriction endonuclease is *SapI*.

9. The method of claim 1, wherein the nucleic acid molecule template is selected from the group consisting of genomic DNA, cDNA, vector DNA, and a chemically-synthesized nucleic acid molecule.

10. The method of claim 2, wherein at least one nucleic acid molecule template is selected from the group consisting of genomic DNA, cDNA, vector DNA, and a chemically-synthesized nucleic acid molecule.

11. The method of claim 1, wherein each of the amplified products comprises at least a portion of an exon.

12. The method of claim 1, wherein each of the amplified products comprises a nucleotide sequence capable of controlling gene expression.

13. The method of claim 12, wherein each amplified product comprises at least one of a regulatory element and a promoter.

14. The method of claim 13, wherein the regulatory element is an enhancer.

15. The method of claim 1, wherein at least one of the amplified products comprises at least a portion of an exon, and at least one of the amplified products comprises a nucleotide sequence capable of controlling gene expression.

16. The method of claim 1, wherein the continuous nucleotide sequence of interest encodes an amino acid sequence, and wherein each of the amplified products comprises an exon.

17. The method of claim 16, wherein one primer of each primer pair is partially complementary to the antisense strand of the 5' end of an exon, and wherein the other primer of each primer pair is partially complementary to the sense strand of the 3'-end of the exon.

18. The method of claim 1, wherein at least one of the amplified products comprises at least one mutation of the nucleotide sequence, which resides in the corresponding nucleic acid molecule template.

19. The method of claim 18, wherein at least one mutation resides in an amino acid encoding sequence.

20. The method of claim 1, wherein the act of amplification is performed using a polymerase chain reaction.

21. A nucleic acid molecule obtained by the process of claim 1, wherein the nucleic acid molecule is produced by ligating 2 to 20 nucleic acid molecule fragments.

22. The nucleic acid molecule of claim 21, wherein the nucleic acid molecule is produced by ligating two nucleic acid molecule fragments.

23. A nucleic acid molecule obtained by the process of claim 1, wherein the nucleic acid molecule is produced by ligating greater than 20 nucleic acid molecule fragments.